Use of Whole Slide Imaging in Nonclinical Toxicology Studies: Questions and Answers

Guidance for Industry

DRAFT GUIDANCE

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April 2022
Pharmacology/Toxicology

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Use of Whole Slide Imaging in Nonclinical Toxicology Studies: Questions and Answers Guidance for Industry[1]

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I. INTRODUCTION

This guidance provides information to sponsors and nonclinical laboratories regarding the use and management of whole slide images used during histopathology assessment and/or pathology peer review performed for good laboratory practice (GLP)-compliant nonclinical toxicology studies using non-human specimens.[2] When whole slide imaging is used as part of a nonclinical study conducted in compliance with the GLP regulations, adequate documentation is critical. The FDA's expectations regarding documentation practices during generation, use, and retention of whole slide images have not been clearly defined and vary among nonclinical testing facilities. This question-and-answer document is intended to clarify FDA's recommendations concerning the management, documentation, and use of whole slide imaging in histopathology assessment and/or pathology peer review for nonclinical studies conducted in compliance with the GLP regulations.

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way, unless specifically incorporated into a contract. This document is intended only to provide clarity to the public regarding existing requirements under the law. FDA guidance documents, including this guidance, should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

[1] This guidance has been prepared by the Office of Study Integrity and Surveillance in the Center for Drug Evaluation and Research in cooperation with the Center for Biologics Evaluation and Research, Center for Devices and Radiological Health, Center for Veterinary Medicine, Center for Food Safety and Applied Nutrition, Center for Tobacco Products, and Office of Regulatory Affairs at the Food and Drug Administration.

[2] We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if it they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method.

II. BACKGROUND

The histopathological assessment of tissue samples is one of the key activities conducted during GLP-compliant nonclinical laboratory studies. Commonly, the histopathological assessment includes an initial evaluation of glass histology slides[3] by the study pathologist and a subsequent review (referred to as pathology peer review) by a second pathologist, group of pathologists, or Pathology Working Group. When whole slide imaging is used as part of a nonclinical study conducted in compliance with the GLP regulations (21 CFR Part 58), the management, documentation, and use of whole slide images in histopathology assessment and/or pathology peer review should be clear and follow written processes and procedures.

Use of whole slide images in casual consultations, opinion exchanges, and mentoring among pathologists is not covered by this guidance document.

III. QUESTIONS AND ANSWERS

Q1: What is whole slide imaging?

A1: Whole slide imaging includes the software and hardware used to generate a two-dimensional digital image[4] of a glass histology slide used for routine assessment in generation of the pathology report. The process includes four sequential parts: image acquisition (scanning), image processing, image file storage, and display of images. Due to inherent limitations of the current technologies used in the process that digitalizes the spatial and color information from the scanned histology slides, FDA does not consider the resulting digital image to be an exact copy of the glass slide. For example, the scanning systems have limited spatial and color resolution and loss of depth of field.[5]

Q2: Should whole slide image files be retained?

A2: If whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies, the whole slide image files should be retained as study records and archived after study finalization. Consideration should be given to ensure that archived digital images remain viewable as software/hardware updates/versions are implemented.

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[3] In the context of this guidance, the term histology slide refers to tissue mounted on a microscope slide, including organ sections and cell samples such as bone marrow and other cytological preparations.

[4] Digital images comprise a sequence of small images (referred to as tiles) taken from distinct locations on the glass slide. Whole slide imaging systems typically determine the optimal focal plane at a limited, discrete set of locations on the glass slide and interpolate the optimal focal plane to generate all of the tiles. The individual tiles are then combined to create the "whole slide" image.

[5] Guidance for Industry and Food and Drug Administration Staff *Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices* (April 2016), section IV (A)(6) *Image Processing Software* and section IV (A)(7) *Image Composition*. We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

Q3: If the whole slide image files are retained, should the glass slides also be retained?

A3: Yes. The glass slides are study specimens and must be retained as study specimens after study finalization in accordance with 21 CFR Part 58.

Q4: What should be retained with respect to the whole slide image file? Should modified whole slide image files be retained?

A4: The whole slide image files assessed by the pathologist for histopathology assessment and/or pathology peer review (i.e., files containing all image data captured by the sensor and documentation of any modifications), referred to here as the original whole slide image files, should be retained. Specifically, any technical image processing modifications made to whole slide image files prior to being provided to the pathologist (e.g., smoothing, color manipulation) should be documented and retained. Viewing software should not allow the original whole slide image files to be changed. Simple adjustments made by the pathologist using the image viewing software during whole slide image evaluation (e.g., brightness, contrast) do not need to be documented or retained.

Q5: Should written procedures for whole slide imaging processes be in place?

A5: Yes, written procedures for whole slide imaging processes should be in place. These may include slide scanning, validation, training, maintenance, software version control, backup/disaster recovery, virus protection, archival, secure access controls, and chain of custody processes.

Q6: Should the whole slide imaging system be validated?

A6: If the whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies, the whole slide imaging system (including software and hardware) should be validated and maintained in a manner specific to the intended use of the technology, consistent with 21 CFR Part 58.

Q7: How should whole slide image files be protected, including when transmitted to external users?

A7: If the whole slide images are assessed in lieu of the original glass slides during histopathology assessment and/or pathology peer review performed for GLP-compliant nonclinical toxicology studies, generation of a backup file, chain of custody, access controls, and securing data systems and data transmission should be performed following written procedures and processes in compliance with an electronic record under 21 CFR Part 11 to maintain whole slide image file integrity.

Q8: Should the signed pathology report/peer review statement state that whole slide images were evaluated in lieu of glass slides?

A8: Yes, the signed pathology report should state whether the glass slides or whole slide images were used for histopathological evaluation by the study pathologist, consistent with 21 CFR 58.185(a). If a pathology peer review is performed, the pathology peer review statement should indicate whether whole slide images or glass slides were reviewed.[6]

[6] Guidance for Industry *Pathology Peer Review in Nonclinical Toxicology Studies: Questions and Answers* (December 2021).